Monitoring of the photochemical degradation of metamitron and imidacloprid by micellar electrokinetic chromatography and differential-pulse polarography

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Abstract: The photochemical degradation of metamitron (4-amino-4,5-dihydro-3-methyl-6-phenyl-1,2,4-triazin-5-one) and imidacloprid (1-(6-chloro-3-pyridylmethyl)-*N*-nitroimidazolidin-2-ylideneamine) has been investigated by differential-pulse polarography (DPP) and micellar electrokinetic chromatography (MEKC); the degradation pathways of these pesticides were elucidated and their degradation products proposed.

The electrochemical study of imidacloprid by DPP at different pH values demonstrated the occurrence of two different reduction processes; at pH 6.8, two peaks at −0.90 V and −1.38 V, respectively, were obtained, which are related to the photochemical reduction processes. The photochemical degradation of imidacloprid caused by sunlight was polarographically monitored and its degradation products elucidated. The polarographic reduction of deaminometamitron (obtained by photochemical reduction of metamitron) yielded two peaks at −0.62 and −1.37 V, which are related to the reduction of the C=N bonds. The effect of sunlight on the reduction of metamitron was monitored by DPP, and an increase of the concentration of the degradation products was observed with time.

MEKC with UV-visible detection was used to separate the pesticides and the products of their photochemical degradation, which were identified in combination with DPP.

Keywords: Capillary electrophoresis; differential-pulse polarography; metamitron; imidacloprid; photochemical degradation

1 INTRODUCTION

The broad use of pesticides in agriculture has increased their levels in the environment. Moreover, the degradation mechanisms of pesticides are not always well understood, and many degradation products have still to be identified.

Metamitron and imidacloprid are pesticides widely used in crops of Castilla y León (Spain). Metamitron (4-amino-4,5-dihydro-3-methyl-6-phenyl-1,2,4-triazin-5-one) is a triazine herbicide used in pre-sowing and in pre- and post-emergence treatment of sugar beet crops, to control broad- leaf plants by interfering with their photosynthesis cycle. Imidacloprid (1-(6-chloro-3-pyridylmethyl)-*N*-nitroimidazolidin-2-ylideneamine) is a systemic and contact insecticide employed against sucking insects such as aphids, leafhoppers and plant hoppers, thrips or whiteflies. This insecticide is specially appropriate for seed treatment and soil application in a variety of crops, such as maize, potato and beet.[1]

Metamitron readily adsorbs onto clay/humic colloids and may persist from one to four months in the soil. Sunlight has been demonstrated to cause degradation of metamitron into deaminometamitron,[2] some research has been carried out on the factors influencing the degradation of imidacloprid in plants and soils.[3,4]

Electrochemical techniques such as polarography and voltammetry are useful to identify and quantify many pesticide compounds,[5] and to elucidate their degradation mechanisms; a variety of herbicide and pesticide compounds, including organochlorine, organophosphorus and nitro-containing compounds, have been investigated by these means.[6–13] The electrochemical reduction of metamitron has been investigated by several authors[14–17] who propose different reduction pathways. This disagreement shows the difficulty in elucidating the degradation mechanisms of pesticides.

Capillary electrophoresis in the micellar electrokinetic chromatographic mode (MEKC) has been widely used to separate and detect a variety of

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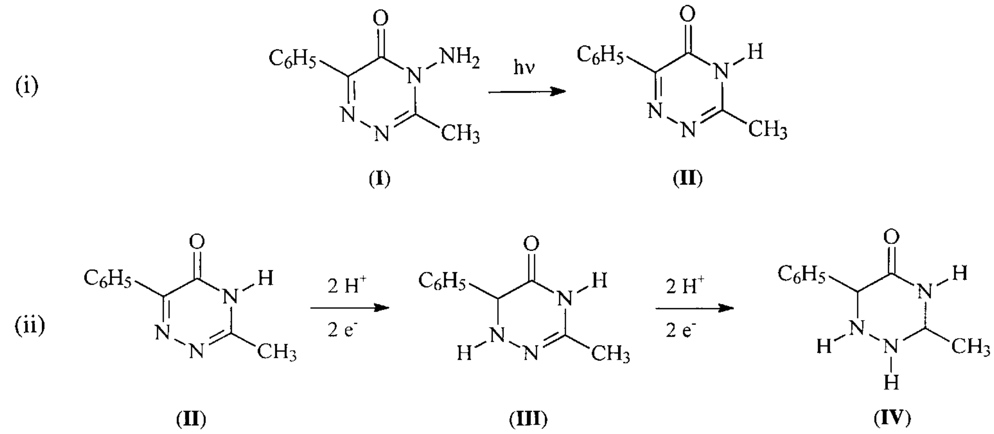

Figure 1. Degradation of metamitron (**I**) and deaminometamitron (**II**).

pesticides and their metabolites.[18–25] In this paper, a MEKC procedure for the separation of the herbicide metamitron, the insecticide imidacloprid, and the products of their photochemical degradation is described and applied to aqueous samples. The degradation products obtained by photochemical reduction of aqueous solutions of these pesticides can be identified in combination with differential-pulse polarography (DPP).

2 MATERIALS AND METHODS

Polarographic measurements were performed with a Metrohm E-506 polarograph (Metrohm, Herisau, Switzerland) in combination with a Metrohm 663VA stand equipped with a static mercury drop electrode (SMDE), an Ag/AgCl, $KCl_{(sal)}$ reference electrode and a platinum auxiliary electrode. pH measurements were made with a Metrohm 654 pH-meter (Metrohm, Herisau, Switzerland). Separation of pesticides and their degradation products was performed by micellar electrokinetic chromatography with a Beckman P/ACE System 5500 capillary electrophoresis instrument (Beckman, Palo Alto, CA, USA), using a fused-silica capillary 50 cm long (from injection point to detector) and 75 μm ID thermostated at 25 °C. The applied voltage was 10 kV, and the intensity current 15 μA; spectrophotometric detection at 214 nm was used.

Metamitron (70%) was obtained from Bayer (Leverkusen, Germany) and used after purification by recrystallization from dichloromethane up to 99.8%. Imidacloprid of a certified purity of 99% was purchased from Riedel-de-Haën (Seelze, Germany) and used without further purification.

Acetate buffer (0.1 M, pH 4.6) and phosphate buffer (0.1 M, pH 6.8) were prepared from analytical-grade reagents, and used for polarographic measurements. The separation buffer used in MEKC analysis consisted of a mixture of 50 mM borate buffer (pH 8.5), 22 mM sodium dodecylsulphate (SDS) and 100 ml litre^{-1} methanol in water.

3 RESULTS AND DISCUSSION

3.1 Electrochemical reduction

An investigation into the electrochemical reduction of metamitron and imidacloprid was carried out by differential-pulse polarography. Polarography was selected as it combines some advantages: (i) the degradation reaction is produced *in situ*, (ii) the degradation products are formed on the electrode

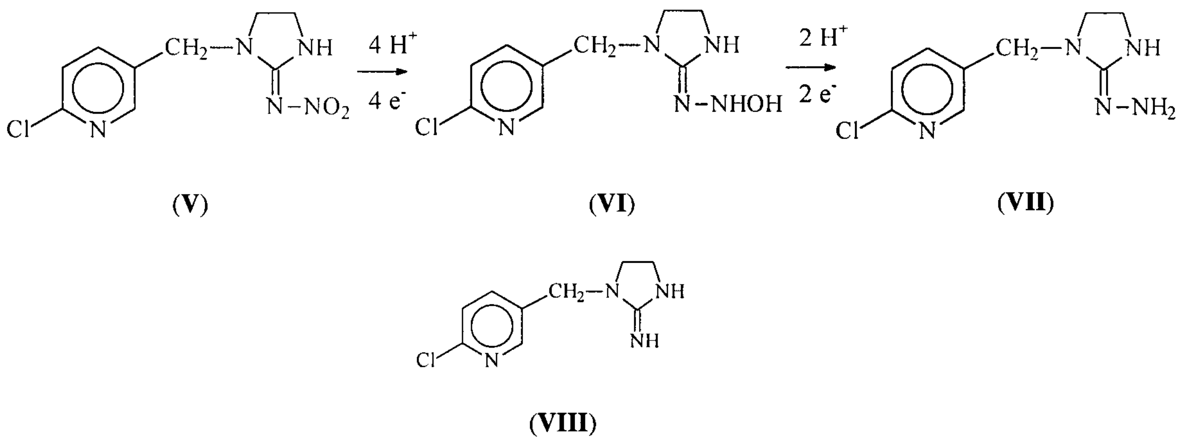

Figure 2. Degradation of imidacloprid (**V**).

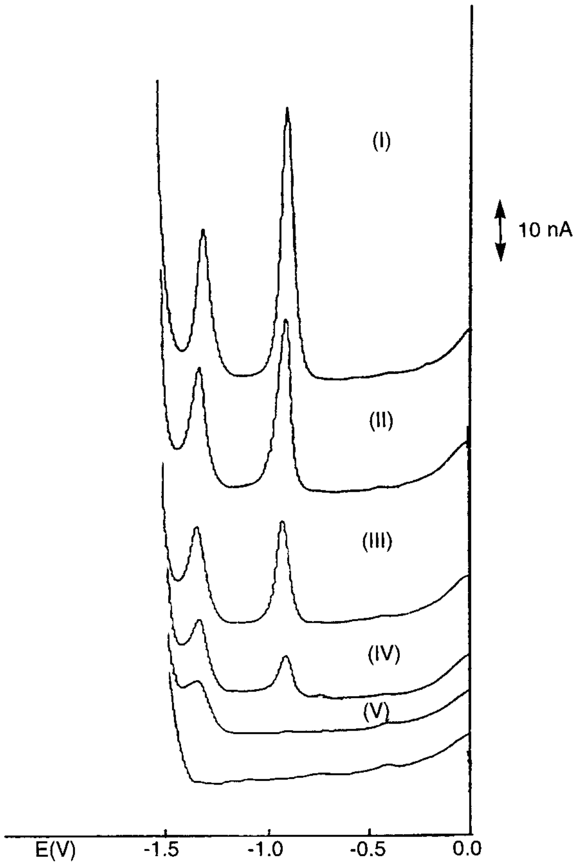

Figure 3. DPP monitoring of the photochemical degradation of an aqueous solution of 200 mg litre^{-1} imidacloprid exposed to sunlight. Aliquots were analysed at different times in phosphate buffer (pH 6.8). (I) 0 days, (II) 15 days, (III) 23 days, (IV) 36 days, (V) 46 days.

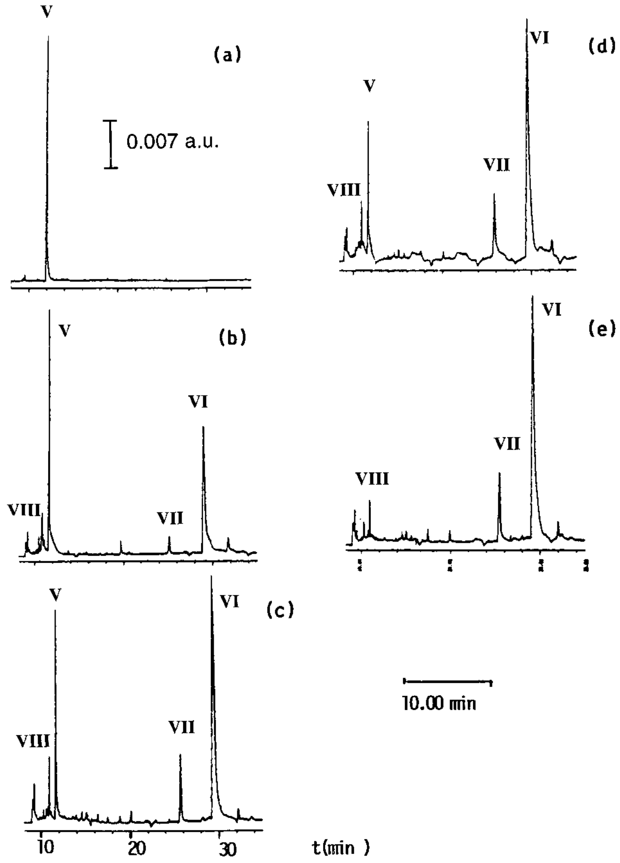

Figure 5. Photochemical degradation of 200 mg litre^{-1} imidacloprid in aqueous solution determined by MEKC. (a) 0 days, (b) 15 days, (c) 23 days, (d) 36 days, (e) 46 days of exposure to sunlight.

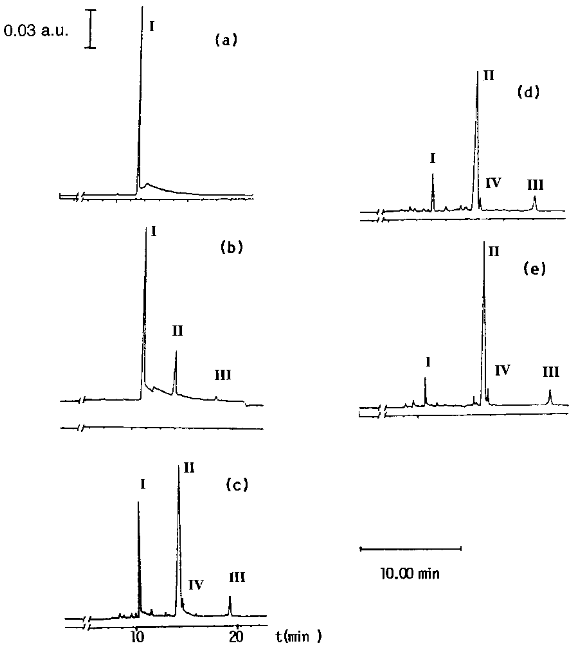

Figure 4. MEKC electropherogram of metamitron and the products of its photochemical reduction. An aqueous solution of 500 mg litre^{-1} metamitron was exposed to sunlight in a quartz container, and aliquots were injected after (a) 0 days, (b) 2 days, (c) 9 days, (d) 14 days, and (e) 21 days of exposure.

surface and are immediately analysed, thus allowing the identification and investigation of less stable species and preventing further transformation of the compound, and (iii) no chemical products need to be added, thus facilitating the identification of species and avoiding the occurrence of side reactions.

A previous study devoted to the electrochemical characteristics of metamitron[15] made it possible to establish the optimal experimental conditions for the qualitative and quantitative analysis of this herbicide. Because it has been demonstrated that light causes the degradation of metamitron into deaminometamitron (3-methyl-6-phenyl-1,2,4-triazin-5-one),[2] the polarographic reduction of this last compound was investigated. A standard solution of 500 mg litre^{-1} metamitron (I) was exposed to sunlight in a quartz tube for one month in order to reduce the pesticide quantitatively into deaminometamitron (II). The completeness of the reduction reaction was verified by DPP and gas chromatography-mass spectrometry (GC-MS) as described in a previous work.[16] The photochemical reduction can be schematised as in Fig (1)

The irradiated solution was used to investigate the reduction of deaminometamitron by DPP in the same conditions used for metamitron.[5,16] A solution of 1 mg litre^{-1} deaminometamitron in 0.1 M acetate buffer (pH 4.6) was polarographically reduced, yielding two

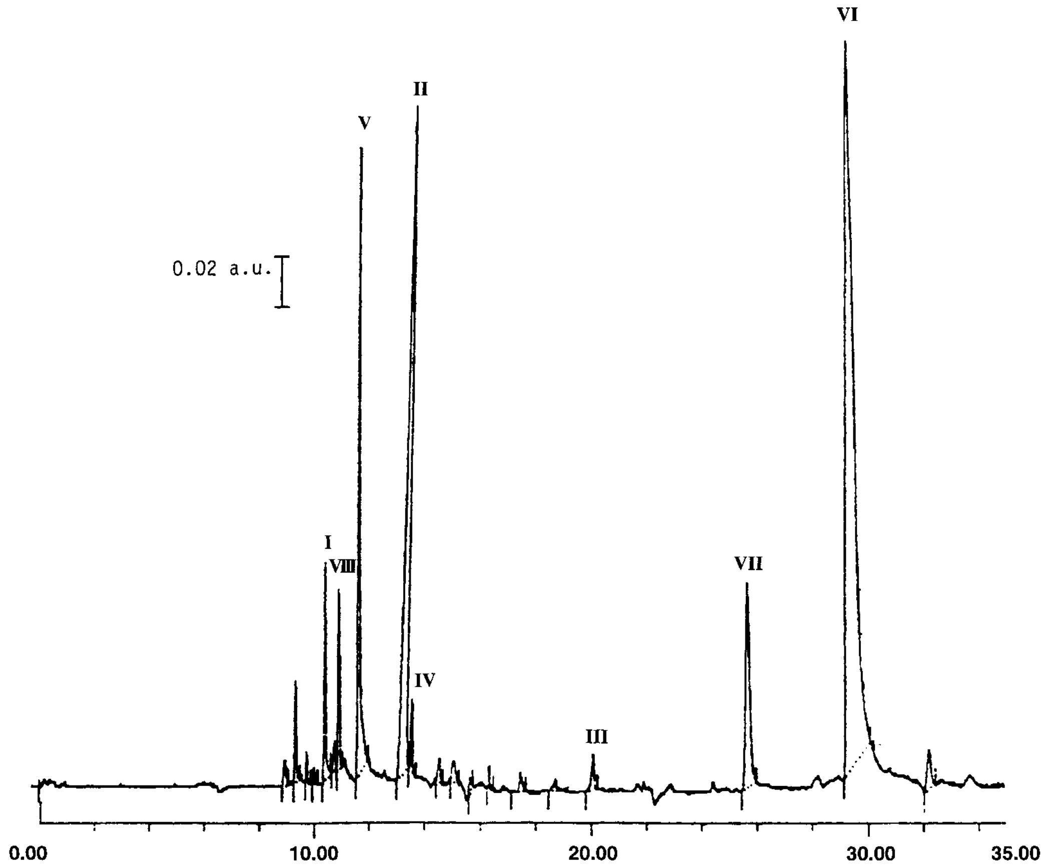

Figure 6. MEKC electropherogram of an aqueous solution of 125 mg litre^{-1} metamitron and 200 mg litre^{-1} imidacloprid showing the degradation products after three weeks of exposure to sunlight.

reduction peaks: a first peak located at -0.62 V is due to the reduction of the C=N bond in position 1,6 into HC-NH, yielding the compound III. The peak at -1.36 V corresponds to the reduction of the C=N bond in position 2,3 to give the compound IV. The reduction pathway of deaminometamitron can be therefore written as in Fig 1 (ii)

The polarographic study of imidacloprid was carried out at different pH values, using a variety of supporting electrolytes which simultaneously set the pH and the ionic strength. Aliquots of the different electrolytes were spiked with a stock solution of imidacloprid up to a concentration of 1 mg litre^{-1}, purged with nitrogen for 10 min to remove dissolved oxygen, and the polarogram registered by DPP. At pH 6.8 ($H_2PO_4^{-}$/HPO_4^{2-} buffer) a first peak at -0.90 V related to the reduction of the —NO_2 group into —NHOH,[26] and a second one at -1.38 V associated with the reduction of NHOH into NH_2[26] were obtained. The electrochemical process can hence be written as in Fig 2.

3.2 Photochemical degradation

The influence of sunlight on the degradation of metamitron was studied by DPP in acetate buffer in a previous work:[16] aqueous solutions of 500 mg litre^{-1} metamitron were exposed to sunlight in quartz tubes, and aliquots of the solution were monitored every few days for one month. It was verified that metamitron is first reduced to deaminometamitron (**II**) by loss of the amino group in position 4. The reduction is completed within one month, as demonstrated by the absence of the reduction peak at -1.25 V (which corresponds to the reduction of the N-NH_2 group into N-H). Deaminometamitron is further reduced to **III** with time, but this reduction is not quantitative within one month as the presence of two reduction peaks at -0.62 and -1.36 V suggests (see above).

The effect of sunlight on the degradation of imidacloprid has been also investigated by DPP. Aqueous solutions of 200 mg litre^{-1} imidacloprid were exposed to sunlight in quartz tubes; an identical solution was protected from UV-visible irradiation and used as reference. The polarographic curves were obtained as described above; the solution exposed to sunlight showed that the peaks at -0.90 V and -1.38 V decrease with time, corresponding respectively to the reduction of the group N-NO_2 (compound **V**) and the reduction of the group N-NOH (compound **VI**) (Fig 3).

3.3 Determination by mlcellar electrokinetic chromatography

Aqueous samples of the herbicide metamitron and the insecticide imidacloprid and the metabolites generated by photochemical degradation were analysed by capillary electrophoresis in the MEKC mode, using a fused-silica capillary of 50 cm (from injection point to detector) and 75 μm ID), [1] at a constant temperature

of 25°C; the applied voltage was 10 kV and the intensity of current 15 μA; samples were injected under a constant pressure of 0.5 psi for 10 s. The electrolyte buffer consisted of 50 mM borate buffer (pH 8.5), 22 mM SDS and 100 ml litre^{-1} methanol. The detection wavelength was set at 214 nm.

The photochemical reduction of these pesticides was followed simultaneously by DPP and MEKC in order to assign the MEKC signals to the different degradation products.

Figure 4 shows the electropherograms corresponding to the photochemical degradation of metamitron with time when a 500 mg litre^{-1} solution was exposed to sunlight in a quartz tube. The progressive reduction of metamitron, (**I**) (10.43 min), into deaminometamitron (**II**) (13.62 min) can be observed. This becomes predominant after nine days, and its subsequent degradation yields small amounts of the compounds **III** (20.09 min) and **IV** (14.05 min) that are observed after two and nine days of sunlight exposure, respectively.

Figure 5 displays the temporal evolution of the electropherograms registered for a solution of 200 mg litre^{-1} imidacloprid exposed to sunlight. As in the case of metamitron, the signal of imidacloprid, (**V**) (11.65 min) decreases with time, thus indicating its progressive degradation into the compounds identified by DPP and labelled as (**VI**) (29.31 min) and (**VII**) (25.67 min). The degradation of imidacloprid is completed within 46 days, as the absence of the MEKC peak at 11.65 min in Fig 5(e) suggests. The degradation product **VI** then becomes predominant, and significant amounts of **VII** are identified. However, this compound is found not to increase significantly with time, thus pointing to its possible reduction to compound **VIII** (10.92 min), by loss of the amino group. This compound was not registered by DPP, probably because the reduction of **VII** into **VIII** takes place at potentials more negative than that of the reduction of the medium.

An aqueous solution containing 125 mg litre^{-1} metamitron and 200 mg litre^{-1} imidacloprid was exposed to sunlight in a quartz cell for three weeks. The solution was then injected into the MEKC instrument and the electropherogram registered (Fig 6). The peaks of the pesticides and their degradation products can be observed, thus allowing the simultaneous identification of all these species in the mixture.

In conclusion, the simultaneous use of electrochemical and separation techniques in pesticide research allows the identification of degradation products and the elucidation of the degradation pathways of pesticides.

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